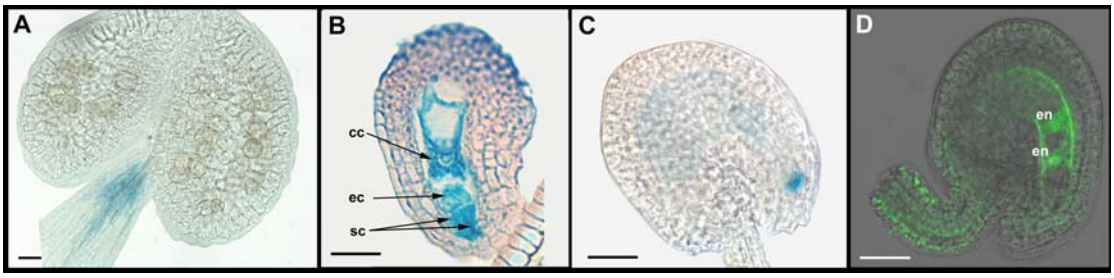
Supplemental data. Bemer et al. (2008). The MADS domain protein DIANA acts together with AGAMOUS-LIKE80 to specify the central cell in *Arabidopsis* ovules.



Supplemental Figure 1: *DIA* expression analysis using *pDIA*:*GFP-GUS* and *pDIA:DIA-GFP-GUS* reporter lines

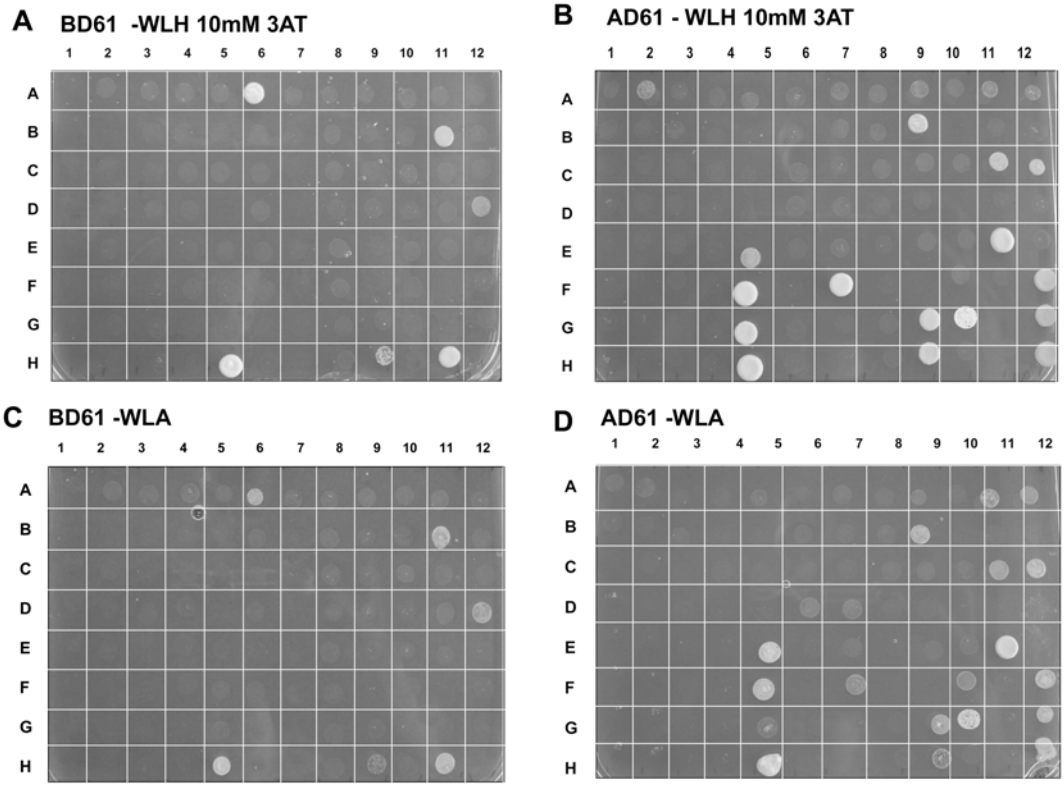
(A) GUS expression under control of the *DIA* promoter (*pDIA*:*GFP-GUS*) in filaments just before anthesis of the anther.

(B) 7.0 µm technovit section of a GUS-stained ovule at stage FG7. GUS expression under control of the *DIA* promoter is visible in the synergid cells (sc), the egg cell (ec) and the central cell (cc).

(C) GUS expression under control of the *DIA* promoter in developing seeds 24 hours after pollination.

(D) GFP signal in the first two endosperm nuclei (en) of a p*DIA*:*DIA*-GFP-GUS ovule 10 hours after pollination.

Bars are 40 µm.



E AD vectors

	1	2	3	4	5	6	7	8	9	10	11	12
A	PI	5	13	21	29	38	46	54	62	72	79	87
B	AP3	6	14	22	31	39	47	55	63	73	80	88
C	AG	7	15	23	32(I)	40	48	56	64	74N	81	89
D	1	8	16	24	32(II)	41	49	57	65	74(I)	82	90
E	2	9	17	25	34	42	50	58	66	74(II)	83	91
F	3(I)	10	18	26	35	43	51	59	x	76	x	92
G	3(II)	11	19	27	36	44	52	60	68	77	85	93
H	4	12	20	28	37	45	53	61	71	78	86	96

F BD vectors

	1	2	3	4	5	6	7	8	9	10	11	12
A	PI	6	15	23	32(I)	40	48	56	65	74(I)	82	90
B	AP3	7	16	24	32(II)	41	49	57	66	74(II)	83	91
C	AG	8	17	25	34	42	50	59	67	76	84	92
D	1	10	18	26	35	43	51	60	68	77	85	93
E	3(I)	11	19	27	36	44	52	61	71	78	86	96
F	3(II)	12	20	28	37	45	53	62	72	79	87	97
G	4	13	21	29	38	46	54	63	73	80	88	98
H	5	14	22	31	39	47	55	64	74N	81	89	99

Supplemental Figure 2. Yeast two-hybrid interaction assay

Protein-protein interaction data of AGL61 (DIA) as bait (**A** and **C**) and as prey (**B** and **D**) against the set of Arabidopsis MADS box prey and bait vectors constructed by de Folter et al. (2005). The position of the different MADS box prey and bait vectors on the plates is shown in tables **E** and **F**. The numbers stand for the different *AGAMOUS-LIKE* genes (e.g. 86 = *AGL86*). The combined yeast cultures were grown on selective SD medium without Leu, Trp and His supplemented with 10mM 3AT (**A** and **B**) or on selective SD medium without Leu, Trp and Ade (**B** and **C**).

The plates were incubated at 20^0C and the growth was scored after four days. The pictures were taken after six days of incubation. The combinations with PHERES1 (AGL37), PHERES2 (AGL38), AGL80 and AGL86 were scored as positive interactions on all plates. Several combinations showed growth on the plates with the set of MADS box factors as bait and AGL61 as prey, but not on the reciprocal plates. For most of the combinations (with AGL99, AGL98, AGL97, AGL92, AGL84, AGL66, AGL73, AGL74N, and AGL39), this is due to auto-activation of the baits and it does not result from interaction with AGL61 (Richard Immink, personal communication; de Folter et al., 2005). However, AGL53 has not been shown to have auto-activation and the growth of this combination may thus reveal a real interaction with AGL61.